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The Crotylation Way to Glycosphingolipids: Synthesis of Analogues of KRN7000

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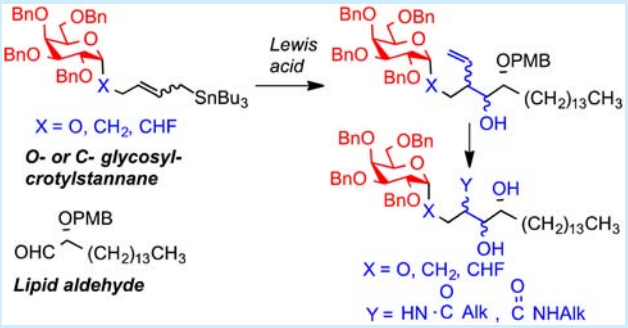
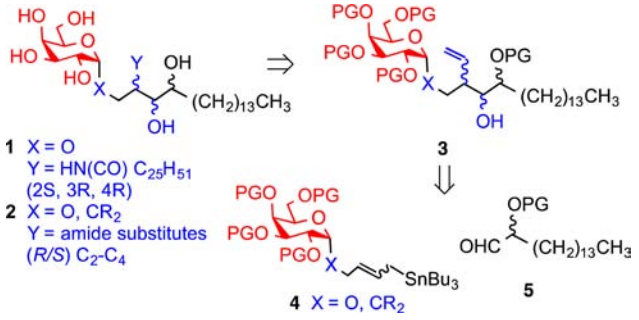
Supporting Information

ABSTRACT: A synthesis of glycosphingolipids that centers on the reaction of *O*- and *C*-glycosyl crotylstannanes and relatively simple lipid aldehydes is described. The modularity of this strategy and versatility of the crotylation products make this an attractive approach to diverse, highly substituted libraries. The methodology is applied to analogues of the potent imunostimulatory glycolipid KRN7000, including O-, methylene-, and fluoromethine-linked isosteres with diastereomeric ceramide segments and 2-amido substitutes.



The presentation of glycolipids typified by KRN7000 **1** by the CD1 family of antigen presenting molecules to invariant natural killer T (iNKT) cells stimulates the production of cytokines that lead to differentiation of T helper cells into Th1 or Th2 cells (Scheme 1).[1] This polarization

Scheme 1. Crotylation Way to Glycosphingolipids



results in an inflammatory or immunomodulatory response. Understanding the mechanism of Th1/Th2 balance is a keynote issue that has ramifications on new therapies for a variety of diseases, including cancer and certain autoimmune disorders.[2] Thus, glycolipids with Th1 or Th2 bias are in demand both for elucidating the molecular basis for cytokine bias and as therapeutic leads. In this context the synthesis and cytokine profiling of novel diverse analogues of KRN7000 has attracted much attention.[3] The polar part of the sphingosine residue has come under close scrutiny, as subtle alterations in this segment have resulted in very different Th1/Th2 profiles. This behavior may be linked to the intimate interactions of this region within the glycolipid-CD1d-TCR ternary complex.[4] Among the more well-known analogues of this type are *O*-and *C*-glycosides with C2−C3 modifications, including different degrees of hydroxylation, stereochemistry, amide replacements, and fluorine substituents.[5−15] Against this backdrop, toward a more rigorous SAR examination of the polar sphingosine segment, we envisaged a synthesis of KRN7000 analogues of the type **2** that centered on the reaction of carbohydrate-derived crotylstannanes **4** with relatively simple aldehydes **5** and elaboration of the crotylation products **3** (Scheme 1).

While crotylations with sugar-derived crotylstannanes and aldehydes have been documented, the potential of this reaction for glycomimetic synthesis has not been fully tapped.[16,17] Unlike more conventional approaches to glycomimetics, this strategy does not focus on the creation of the glycoside (or pseudoglycoside) bond, but on other linkages, thereby avoiding complications with challenging glycoside isosteres, while opening entry to new glycomimetic space.

For the preliminary test of this methodology, tin-based reagents were chosen because of their ease of preparation and greater stability compared to other classes of crotylating agents (Scheme 2).[18,19] Thus, **7**, **10**, and **13** were prepared following the studies by Jarosz, from allylic alcohol precursors **6**, **9**, and **12** respectively.[17] For **7**, **6**[20] was first converted to the xanthate derivative. Thermal rearrangement of the latter and *in situ* treatment of the resulting secondary dithiocarbonate with Bu_3SnH in the presence of AIBN gave crotyltin **7** with an *E*:*Z* ratio of 3:2, in 57% overall yield from **6**. For **10** and **13**, allylic alcohols **9** and **12** were prepared from known alkenes **8**[21] and **11**[22] respectively via standard chain extension−Wittig olefination reaction sequences (Supporting Information). Application of the stannylation protocol to **9** and **12** yielded **10** and **13** as

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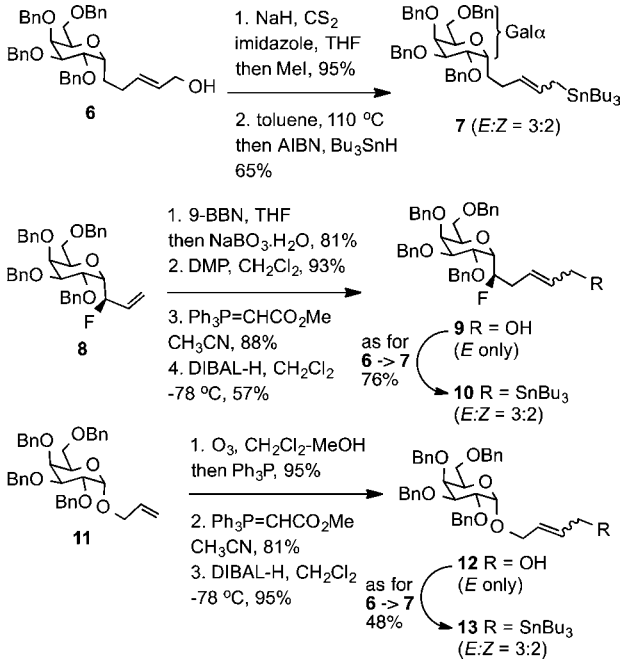
Scheme 2. Synthesis of Crotyltins



3:2 *E:Z* mixtures. The crotyltins were purified using standard extraction and chromatography procedures and could be stored in the refrigerator for several months without decomposition. The *E:Z* isomers were not easily separable and used as mixtures in the subsequent crotylation reactions.

Crotylation reactions were first performed using $BF_3{\cdot}OEt_2$ as catalyst (Table 1). Addition of *E:Z* mixtures of **7**, **10**, and **13** to a preincubated mixture of aldehyde **14**[10] and $BF_3{\cdot}OEt_2$ in dichloromethane at −78 °C afforded diastereomeric mixtures **15a−c**, **16a−c**, and **17a−c** in 86%, 70%, and 70% yields. These reactions were generally completed within 2 h.

Where determined the stereochemistry of crotylation products was assigned in cyclic derivatives and by chemical correlation (*vide infra*). Unreacted aldehyde and derivatives of the crotylation products showed no indication of epimerization at the α-carbon in the aldehyde during the crotylation reaction. The stereochemical trends of these complex sugar-derived crotyltins are similar to the reactions of simple achiral crotyltins.[18,19,23,24] Thus, the preference for 3,4-*syn* over 3,4-*anti* diastereomers (ca. 7:1 for **7** and 5:1 for **13**) is in line with the observation that both *E* and *Z* reagents generally show good to high preference for 3,4-*syn* adducts. This behavior has been reasoned with open transition state models.[18,19,23,24] A preliminary study on the effect of a Lewis acid on stereochemistry was also performed. Whereas, the $BF_3{\cdot}OEt_2$ promoted reaction of **7** and **14** led primarily to the 3,4-*syn* products **15a** and **15b**, giving exclusively the Cram (i.e., 4,5-*syn*) products **15b** and **15d**, which is expected for such chelating Lewis acids. This erosion in 3,4-*syn* selectivity in changing from the noncoordinating to chelating promoter is also precedented.[24] Together the data with $BF_3{\cdot}OEt_2$ and $MgBr_2{\cdot}OEt_2$ illustrate that Lewis acids could be used to control stereoselectivity in these complex crotylations. Stereochemical control through the use of chiral acid promoters and/or other metalating entities is also envisaged.[18,19,25]

The stereochemistry of crotylation products **15a−d** and **17a−c** were determined by ^{1}H NMR analysis of their derived lactones and chemical correlation. This was achieved via

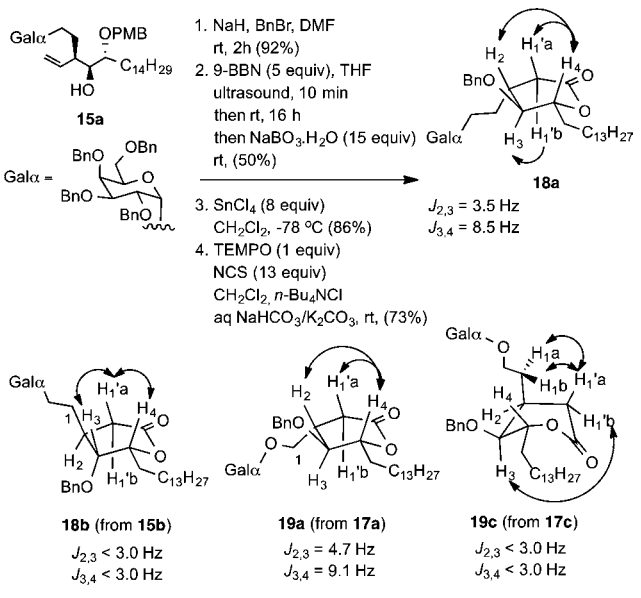
Table 1. Reactions of *C*-Glycoside Crotyltins and Aldehydes

crotyltin	aldehyde	crotylation products[a]
E/Z-7	14	**15a** (38%) : **15b** (37%): **15c** (11%) **15b** : **15d** (75%, rel. ratio 1/1.7)[b,c]
E/Z-10	14	**16a** (51%) : **16b** : **16c** (19%, rel ratio 4/1)[c,d]
E/Z-13	14	**17a** (34%) : **17b** : **17c** (36% rel ratio 1.4/1)[c]

[a]Unless otherwise stated reactions were promoted with $BF_3.OEt_2$; isolated yields of separated products are indicated in parentheses. [b]$MgBr_2{\cdot}OEt_2$ promoted reaction. [c]**15b/d**, **16b/c**, and **17b/c** were isolated as mixtures; diastereomer ratios were determined by NMR. [d]Stereochemistry of products was not assigned.

oxidation−reduction sequences on the original crotylation products (Scheme 3, Supporting Information). Thus, transformation of **15a** to its benzyl ether and hydroboration−oxidation of the alkene afforded the primary alcohol derivative.

Scheme 3. Stereochemical Assignment of Crotylation Products



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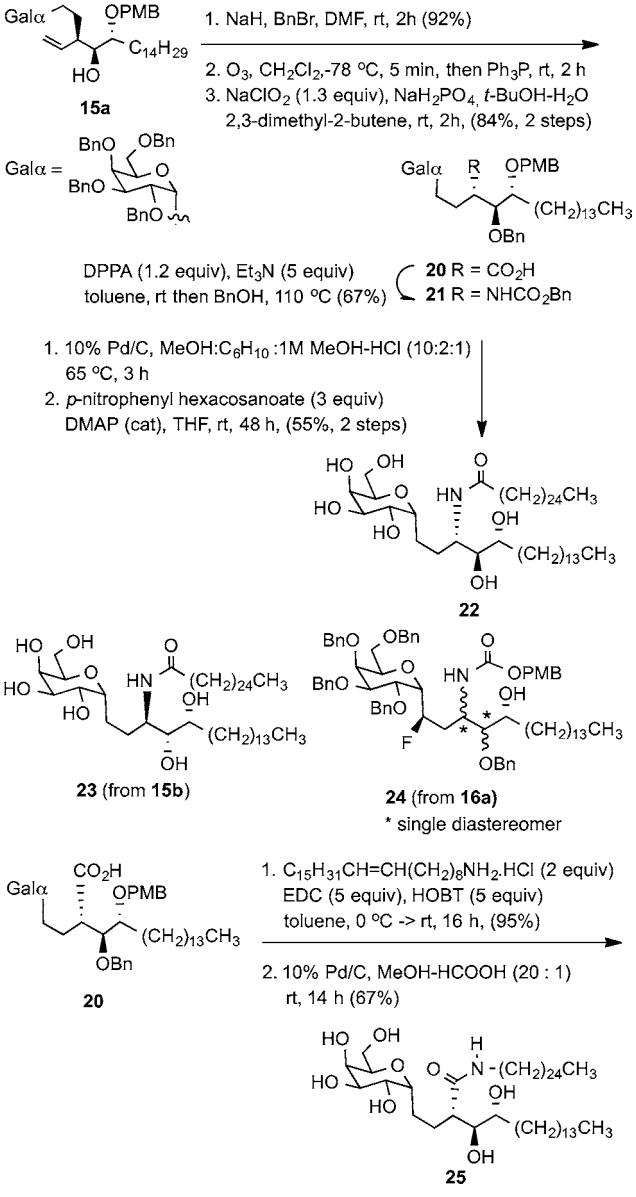
Removal of the PMB ether and oxidation of the resulting diol afforded lactone **18a**. Similar transformations on **15b**, **17a**, and **17c** led to lactones **18b**, **19a**, and **19c** respectively. The stereochemistry of these lactones was deduced from analysis of $J_{H,H}$ values and nOes. The stereochemistry of **15a** was confirmed by transformation to *C*-KRN7000 (*vide infra*).

The syntheses of *C*-KRN7000 **22**, the 2,3-bisepimer of *C*-KRN7000 **23**, the fluorinated *C*-glycoside **24**, and the *C*-KRN7000 analog with an inverse amide **25** provide a glimpse of the diverse sphingolipid analogues that can be prepared via this crotylation methodology (Scheme 4). *C*-KRN7000 **22** is a

Scheme 4. Synthesis of *C*-Glycosphingolipids



validated immunostimulant with superior Th1 bias in mice compared to KRN7000.[6,10] The 2,3-bisepimer of *C*-KRN7000 **23** is of interest because another diastereomer, 4-epi-*C*-KRN7000, exhibits high Th1 cytokine bias and increased potency compared to *C*-KRN7000.[12] Likewise, other diastereomers of *O*- and *C*-KRN7000 have interesting cytokine profiles.[5,9] Fatty acid derivatives of **24** are intriguing because the location and conformational and electronic properties of the fluorine substituent may induce unusual binding in the glycolipid-CD1d-TCR ternary complex, which in turn may elicit unique cytokine activity.[4,15] Similarly the reverse amide **25** is a probe for the binding of the key amide residue.[4]

For *C*-KRN7000 **22**, **15a** was processed to carboxylic acid **20** via routine alcohol protecting group and alkene transformations. A Curtius rearrangement protocol using diphenylphosphorylazide (DPPA) on **20** provided carbamate **21**.[26] Standard conditions for removal of benzyl protecting groups and amine acylation provided **22**, which was determined to be essentially identical by TLC, NMR, and HRMS to an authentic sample.[6,10,11]

Using similar reaction sequences **15b** and **16a** were transformed to **23** and **24** respectively (Supporting Information). This Curtius rearrangement strategy is practical, as *in situ* reaction of the intermediate isocyanate with lipid alcohols, amines, and thiols can provide direct entry to carbamates, thiocarbamates, and ureas of **22** respectively, which are of interest given the cytokine activity of their *O*-linked counterparts.[14] Amidation of acid **20** and pentacos-8-en-1-amine, followed by removal of protecting groups, provided the reverse amide **25**. Straightforward transformations on alkene **15a** to a variety of other novel amide substitutes can be envisaged.

In conclusion, the synthesis of the KRN7000 analogues described herein illustrates the potential of aldehyde crotylation as a strategy for accessing diverse glycosphingolipids. The modular plan, rapid buildup of complexity from easily accessible precursors, and versatility of the crotylation products make this approach appropriate for libraries with different sugar head groups, pseudoanomeric linkages, and ceramide segments. Biological evaluation of these new KRN7000 analogues, further investigations on the stereochemistry of these complex crotylation reactions, and applications of this methodology to other classes of glycomimetics are underway.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b02284.

Experimental procedures and analytical data for new compounds; NMR spectra for selected compounds (PDF)

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Notes

The authors declare no competing financial interest.

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